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## Temporal expression and signalling of prostacyclin receptor in the human endometrium across the menstrual cycle

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### Abstract

Prostacyclin (PGI<sub>2</sub>) synthesis and function in the human uterus has been implicated in the regulation of the process of normal and dysfunctional menstruation. PGI<sub>2</sub> synthesis is elevated during normal menstruation and is also associated with blood loss in women who suffer from heavy menses. This study was designed to outline further the role of PGI<sub>2</sub> in menstruation by investigating the temporal pattern and site of expression of prostaglandin I synthase (PGIS) and the prostacyclin receptor (IP receptor) in the non-pregnant human endometrium across the menstrual cycle. Quantitative RT-PCR demonstrated increased expression of PGIS and IP receptor during the menstrual phase of the cycle compared with all other phases ( $P < 0.05$ ). Furthermore, PGIS and IP receptor were localised to the glandular epithelium, stromal and endothelial cells in the basal and functional layers of the endometrium. Functionality of the IP receptor in the human endometrium was assessed by measuring cAMP generation following treatment with 100 nmol l<sup>-1</sup> of the PGI<sub>2</sub> analogue, iloprost. cAMP generation was significantly higher in endometrial tissue collected during the proliferative compared with the secretory phase of the menstrual cycle ( $P < 0.05$ ).

In conclusion, this study has confirmed increased expression and signalling of PGIS and IP receptor during the menstrual phase and outlines a potential autocrine/paracrine role for PGI<sub>2</sub> on several cellular compartments in the endometrium including the endothelium. This may underscore a pivotal role for PGI<sub>2</sub> receptor signalling in normal and dysfunctional menstruation.

### Introduction

Prostacyclin (PGI<sub>2</sub>) is a member of the prostanoid family of lipid mediators and is the main prostanoid synthesised by vascular endothelium (Smyth & FitzGerald 2002).

Cyclooxygenase (COX) enzyme is the key enzyme in the synthesis of PGI<sub>2</sub> from arachidonic acid via the common intermediate in prostaglandin (PG) synthesis, PGH<sub>2</sub>. In turn, PGH<sub>2</sub> is rapidly converted to PGI<sub>2</sub> by the action of prostaglandin I synthase (PGIS) (Kniss 1999). There are two predominant isoforms of COX enzymes, COX-1, which is constitutively expressed in many cell types and COX-2, which is induced by factors

including cytokines and tumour promoters (Kniss 1999). PGI<sub>2</sub> elicits its effects on target cells by interaction with its G protein-coupled receptor (IP receptor), which has a typical seven-transmembrane structure (Narumiya *et al.* 1999). The IP receptor can stimulate both G<sub>s</sub> and G<sub>q</sub> species of G proteins causing an increase in cAMP generation and in phosphatidylinositol response (Narumiya *et al.* 1999).

Temporal expression of other prostaglandins such as PGE<sub>2</sub> and PGF<sub>2α</sub> and their receptors (EP2/EP4 and FP receptors) has been demonstrated in the human endometrium and has been shown to vary with the phase of the menstrual cycle (Lumsden *et al.* 1986, Smith & Kelly 1988, Milne *et al.* 2001, Milne & Jabbour 2003). Maximal expression and signalling of these receptors are detected during the mid-late proliferative phase of the menstrual cycle. Recent data implicate a role for PGE<sub>2</sub> and PGF<sub>2α</sub> in the proliferation of glandular epithelial cells via diverse signalling pathways (Jabbour & Boddy 2003, Milne & Jabbour 2003). In contrast, little is known of the expression pattern and function of the IP receptor in human endometrium, although PGIS and IP receptor expression have been demonstrated in pregnant and non-pregnant myometrium (Moonen *et al.* 1986, Chegini & Rao 1988, Giannoulas *et al.* 2002).

PGI<sub>2</sub> is the main prostanoid synthesised by the vascular endothelium and it causes blood vessel dilatation and inhibition of platelet aggregation (Smyth & FitzGerald 2002). Moreover, PGI<sub>2</sub> is known to act as a smooth muscle relaxant (Wilhelmsson *et al.* 1981, Lumsden & Baird 1986, Dyal & Crankshaw 1988). The effects of PGI<sub>2</sub> on platelet aggregation and vascular tone have outlined its potential role in menstruation (Baird *et al.* 1996). This is supported by observations of elevated PGI<sub>2</sub> levels in uterine venous blood during menstruation as compared with other phases of the menstrual cycle (Goodfellow *et al.* 1982). Moreover, PGI<sub>2</sub> synthesis is increased in women suffering from heavy menses compared with those who show normal blood loss (Smith *et al.* 1981*b*, Makarainen & Ylikorkala 1986, Cameron *et al.* 1987). It has also been suggested that menstrual disorders can be the result of a shift in the ratio of different prostaglandins. Heavy menses has been associated with increased synthesis of PGI<sub>2</sub> relative to thromboxane A<sub>2</sub> and PGE<sub>2</sub> relative to PGF<sub>2α</sub> (Smith *et al.* 1981*a*, Makarainen & Ylikorkala 1986). Heavy menses with no known uterine pathology affects 10% of women of child bearing age and is defined as blood loss in excess of 80 ml per menstrual cycle (Prentice 2000).

The objectives of the present study were to investigate the expression of PGIS (the terminal enzyme responsible for the generation of PGI<sub>2</sub>) and IP receptor in non-pregnant human endometrium and myometrium across the menstrual cycle. Furthermore, the role of PGI<sub>2</sub> in endometrial cell signalling has been assessed by investigating the effect of the PGI<sub>2</sub> analogue, iloprost, on cAMP generation. A better understanding of IP receptor signalling and function in the human endometrium may ultimately lead to the development of novel therapies in the treatment of menstrual disorders that may be associated with elevated PGI<sub>2</sub> synthesis.

## Materials and Methods

### Patients and tissue collection

Endometrial biopsies at different stages of the menstrual cycle were obtained from women with regular menstrual cycles (25–35 days), who had not received a hormonal preparation in the 3 months preceding biopsy collection. Samples were collected either with an endometrial suction curette (Pipelle, Laboratoire CCD, Paris, France) or as full thickness endometrial biopsies (including the functional layer and basal-myometrial junction) from women undergoing hysterectomy for benign gynaecological indications. Shortly after collection, tissue was either snap frozen in dry ice and stored at –70 °C (for RNA extraction), fixed in

neutral buffered formalin (NBF) and wax embedded (for immunohistochemical analyses), or placed in RPMI 1640 (containing 2 mmol l<sup>-1</sup> L-glutamine, 100 U penicillin and 100 µg ml<sup>-1</sup> streptomycin) and transported to the laboratory for *in vitro* culture.

Biopsies were classified within a 28-day cycle range using stated last menstrual period (LMP) and histological assessment according to the criteria of Noyes and co-workers (1975). Furthermore, circulating oestradiol and progesterone concentrations at the time of biopsy were consistent for both stated LMP and histological assignment of menstrual cycle stage. Samples were divided according to the phase of the menstrual cycle as: menstrual (days 1–4), early to mid proliferative (EP-MP; days 5–10), late proliferative to ovulatory (LP-Ov; days 11–14), early secretory (ES; days 15–18) and mid to late secretory (MS-LS; days 19–28). Ethical approval was obtained from the Lothian Research Ethics Committee and written informed consent was obtained from all subjects before tissue collection.

### Taqman quantitative RT-PCR

RNA was extracted from endometrial biopsies obtained from across the menstrual cycle ( $n = 35$ ) using Tri Reagent (Sigma, Poole, Dorset, UK) following the manufacturer's instructions. RNA samples were quantified and were reverse transcribed using 5.5 mmol l<sup>-1</sup> MgCl<sub>2</sub>, 0.5 mmol l<sup>-1</sup> of each deoxynucleotide triphosphate (dNTP), 2.5 µmol l<sup>-1</sup> random hexamers, ribonuclease inhibitor (0.4 U µl<sup>-1</sup>) and 1.25 U µl<sup>-1</sup> Multiscribe reverse transcriptase (all from Applied Biosystems, Warrington, Cheshire, UK). RNA (400 ng) was added to each reverse transcription reaction and samples were incubated for 90 min at 25 °C, 45 min at 48 °C and 5 min at 95 °C. The reaction mix for the PCR consisted of 1 × mastermix, ribosomal 18S forward and reverse primers, ribosomal 18S probe (50 nmol l<sup>-1</sup>; all from Applied Biosystems), forward and reverse primers for PGIS or IP receptor (300 nmol l<sup>-1</sup>) and PGIS or IP receptor probe (200 nmol l<sup>-1</sup>) (all from Biosource UK, Nivelles, Belgium). The reaction mix (48 µl) was aliquoted into tubes and 2 µl cDNA were added. Duplicate 24 µl samples plus positive and negative controls were placed in a PCR plate and wells were sealed with optical caps. The PCR reactions were carried out using an ABI Prism 7700 (Applied Biosystems). All primers and probes were designed using the PRIMER express program (Applied Biosystems). The sequences of PGIS primers and probe were: PGIS forward primer, 5'-ACGCAGATGTGGAGATCCCT-3'; reverse, 5'-GTCGTGTTCCGGCTGCA-3'; and probe (6-carboxy fluoroscein labelled) 5'-CCTCAGCAGGTACGGCTTCGGTCTG-3'. The sequences of the IP receptor primers and probe were: IP receptor forward primer, 5'-GCCCTCCCCCTCTACCAA-3'; reverse, 5'-TTTTCCAATAACTGTGGTTTTTGTG-3'; and probe (6-carboxy fluoroscein labelled) 5'-CCAAGAGCCAGCCCCCTTCTGC-3'. The sequences of 18S primers and probe have been described previously (Milne *et al.* 2001). Data were analysed and processed using Sequence Detector version 1.6.3 (Applied Biosystems) according to the manufacturer's instructions. Results were expressed relative to an internal positive standard (cDNA obtained from a single sample of endometrial tissue) included in all reactions.

### *In situ* hybridisation

A custom synthesised oligonucleotide double fluoroscein isothiocyanate (FITC)-labelled cDNA probe for IP receptor was obtained from Biognostik (Göttingen, Germany). Sections (5 µm) from full thickness human endometrial biopsies collected across the menstrual cycle ( $n = 12$ ) were cut onto gelatin-coated slides. Sections were dewaxed and rehydrated and then treated with proteinase K (50 µgml<sup>-1</sup> in 100 mmol l<sup>-1</sup> Tris-HCl pH 7.6, containing 50 mmol l<sup>-1</sup> EDTA) for 15 min at 37 °C to enhance cDNA probe access. Sections were washed in diethylpyrocarbonate-treated water and prehybridised for 4 h at 30 °C with 25 µl of the hybridisation buffer supplied with the probe, which had previously been heated to 95 °C. The sections were then hybridised overnight at 30 °C with the cDNA probe at 6 U µl<sup>-1</sup> in

hybridisation buffer. Following hybridisation, sections were washed for  $2 \times 5$  min in  $1 \times$  SSC at room temperature and  $2 \times 15$  min in  $0.1 \times$  SSC at  $39^\circ\text{C}$ . After rinsing in tris buffered saline (TBS), endogenous peroxidase activity was quenched with 10% (v/v)  $\text{H}_2\text{O}_2$  in methanol at room temperature. The FITC-labelled probe was detected using standard immunohistochemical reagents with an additional amplification step (TSA Biotin System, NEN Life Science Products, Hounslow, Middlesex, UK). Sections were incubated with blocking buffer for 30 min. Conjugated anti-FITC-horseradish peroxidase (Roche, Diagnostics Ltd, Lewes, E Sussex, UK) was added at a dilution of 1 in 200 in blocking buffer and the sections were incubated for 30 min. After washing, biotinyl tyramide amplification reagent (1 in 50) was applied to each slide and incubated for 15 min. Streptavidin-horseradish peroxidase (1 in 100) was applied after washing and incubated for 30 min; probe localisation was visualised with 3,3'-diaminobenzidine (DAB) substrate. Control sections were treated with a double FITC-labelled oligonucleotide probe containing the same proportion of cysteine (C) and guanine (G) bases as the IP receptor probe to assess background hybridisation. All treatments were carried out at room temperature unless otherwise specified.

### Immunohistochemistry

Endometrial sections ( $5\ \mu\text{m}$ ) from across the menstrual cycle ( $n = 12$ ) were dewaxed in xylene and rehydrated using decreasing grades of ethanol. Antigen retrieval was performed by treating sections for 5 min in a pressure cooker in boiling 0.1% citrate buffer, pH 3.0. Endogenous peroxidase activity was quenched with 10% (v/v)  $\text{H}_2\text{O}_2$  in methanol at room temperature. Non-immune swine serum (20% serum in TBS) was applied for 1 h before overnight incubation at  $4^\circ\text{C}$  with rabbit anti-human IP receptor at a dilution of 1 in 500 or rabbit anti-bovine PGIS (shown to cross-react with human PGIS) (Alexis Corporation, Nottingham, UK) at the same concentration. An avidin-biotin peroxidase detection system was then applied (DAKO Ltd, Cambridge, UK) with DAB as the chromagen. The antibody to IP receptor has been described previously (Fortier *et al.* 2001). Non-immune rabbit serum and antibody pre-absorbed with IP receptor peptide were used as controls for IP receptor, and non-immune rabbit serum was used as a control for PGIS. Immunoreactivity was negligible with pre-absorbed antibody, and with non-immune rabbit serum there was occasional generalised pale brown cross reactivity over the epithelial glands and the endothelium.

### Cyclic AMP assay

Endometrial biopsies from the proliferative and secretory phases of the menstrual cycle ( $n = 8$ ) were minced finely with scissors and divided into three portions. The tissue was incubated overnight at  $37^\circ\text{C}$  in a humidified 5%  $\text{CO}_2$  incubator in 2 ml RPMI medium (Sigma) containing  $2\ \text{mmol l}^{-1}$  L-glutamine, 100 IU penicillin and  $100\ \mu\text{g}$  streptomycin, and  $3\ \mu\text{gml}^{-1}$  indomethacin (Sigma). Following overnight treatment, the tissue was incubated in the same medium containing 1-methyl-3-isobutylxanthine (Sigma) at  $37^\circ\text{C}$  for 30 min. It was then treated with control medium or  $100\ \text{nmol l}^{-1}$  iloprost (a gift from Schering Health Care, Burgess Hill, W Sussex, UK) for 10 min at  $37^\circ\text{C}$  and lysed in  $0.1\ \text{mol l}^{-1}$  HCl and frozen until assayed. Cyclic AMP concentration was measured by ELISA (Biomol, Affiniti, Exeter, Devon, UK) according to the manufacturer's instructions and normalised to the protein concentration determined by a modification of the method of Lowry (Bio-Rad, Hemel Hempstead, Herts, UK). Data are presented as the fold induction of cAMP after treatment with iloprost, where fold induction was calculated relative to the control samples.

## Statistics

Where appropriate, data were subjected to statistical analysis with ANOVA and Fisher's protected least significant difference tests (Statview 4.0; Abacus Concepts Inc., Piscataway, NJ, USA) and statistical significance was accepted when  $P < 0.05$ .

## Results

The temporal pattern of PGIS mRNA expression in the human endometrium across the menstrual cycle was studied by quantitative RT-PCR. PGIS mRNA was detected in all samples of human endometrium examined (Fig. 1a). Relative expression was significantly higher in the menstrual phase of the cycle compared with all other phases ( $14.49 \pm 5.92$  vs  $2.28 \pm 0.74$  for EP-MP;  $1.25 \pm 0.33$  for LP-Ov;  $1.75 \pm 0.67$  for ES and  $4.7 \pm 3.15$  for MS-LS;  $P < 0.05$  for all comparisons). Similarly, the temporal pattern of IP receptor mRNA expression across the menstrual cycle was assessed by quantitative RT-PCR and IP receptor mRNA was detected in all samples of human endometrium examined (Fig. 1b). Relative expression was significantly higher in the menstrual phase of the cycle compared with all other phases ( $33.94 \pm 17.2$  vs  $2.62 \pm 0.7$  for EP-MP;  $1.36 \pm 0.25$  for LP-Ov;  $2.95 \pm 0.76$  for ES and  $2.87 \pm 0.53$  for MS-LS;  $P < 0.001$  for all comparisons).

Immunohistochemical staining for PGIS was performed in full thickness human endometrial biopsies collected from across the menstrual cycle. A similar pattern of staining was observed in tissue sections from proliferative and secretory phases (Fig. 2 is a representative sample of endometrial tissue collected during the proliferative phase). Cytoplasmic and nuclear immunoreactivity was detected in glandular epithelial cells in the basal (Fig. 2b) and functional (Fig. 2c) layers. Stromal cell reactivity was also present in both layers, but with greater reactivity in the functional than in the basal layer (Fig. 2a). Endothelial cell staining was detected in the microvasculature in both endometrial layers and reactivity was also present in myometrial smooth muscle cells (enlarged area in Fig. 2a).

The site of expression of the IP receptor was studied by *in situ* hybridisation and immunohistochemistry. IP receptor mRNA expression was detected in endometrial samples collected during the menstrual and proliferative phases of the menstrual cycle. IP receptor expression was localised to glandular epithelial and stromal cells in the basal and functional layers of the endometrium (Fig. 3b-d). In addition, expression was present in myometrial smooth muscle cells (Fig. 3a) and in endothelial cells lining vessels in the functional and basal layers of the endometrium and at the basal-myometrial junction (Fig. 3a and c). Immunohistochemical staining for IP receptor protein was detected throughout the menstrual cycle in the cytoplasm and nuclei of glandular epithelial cells in both basal and functional layers (Fig. 4a-c). Similar to PGIS immunoreactivity, IP receptor stromal cell staining was stronger and more widespread in the functional layer (Fig. 4c) compared with the basal layer (Fig. 4b). IP receptor protein expression was also localised to endothelial cells throughout the microvasculature and was present in myometrial smooth muscle cells.

To investigate the functionality of the IP receptor, cAMP generation in response to iloprost (a  $\text{PGI}_2$  analogue) treatment was assessed in endometrial biopsy tissue collected across the menstrual cycle (Fig. 5). Cyclic AMP generation in response to  $100 \text{ nmol l}^{-1}$  iloprost was significantly higher in endometrial samples collected during the proliferative phase compared with the secretory phase ( $4.83 \pm 0.74$  fold increase vs  $2.07 \pm 0.39$  fold increase;  $n = 4$  for each group;  $P < 0.05$ .)



## Discussion

This study demonstrates the expression of PGIS and IP receptor genes in the human endometrium and shows significant up-regulation of both during menstruation. PGIS is the terminal enzyme that leads to synthesis of PGI<sub>2</sub> in target tissues (Kniss 1999). The higher expression level of PGIS during menstruation supports previous observations reporting a temporal pattern of PGI<sub>2</sub> secretion by the human endometrium across the menstrual cycle; PGI<sub>2</sub> concentrations are maximal in uterine venous blood during menstruation (Goodfellow *et al.* 1982). The elevated expression of IP receptor during menstruation suggests that PGI<sub>2</sub> synthesis and IP receptor are temporally regulated to induce their effects on target cells. This is supported by the maximal iloprost-induced cAMP response observed in the endometrium collected during the menstrual/proliferative phases. The IP receptor has been shown to couple to G<sub>s</sub> proteins which are linked to increases in cAMP generation (Narumiya *et al.* 1999). Although only the protein kinase A pathway was investigated in this study, it is predicted that the IP receptor may activate other signalling pathways in the human endometrium, as has been shown recently for other prostaglandin receptors (Jabbour & Boddy 2003, Milne & Jabbour 2003). Such diverse signalling pathways may lead to differential activation of target genes that can promote various phenotypic changes on target cells. In addition to the classical G protein-coupled IP receptor, recent evidence suggests that prostacyclin functions via the nuclear peroxisome proliferator-activated receptor delta (PPAR $\delta$ ) (Lim & Dey 2002). The importance of this mechanism in prostacyclin activity in non-pregnant endometrium is not known. However, it is unlikely to be the predominant signalling pathway, since expression of PPAR $\delta$  in the normal human endometrium is minimal (Tong *et al.* 2000).

The factors that regulate the expression of the PGIS and IP receptor during menstruation in the human endometrium are not clear. It is likely that this temporal expression is regulated by steroid hormones as has been postulated for other prostanoids and their receptors (Milne *et al.* 2001, Milne & Jabbour 2003). Oestradiol-17 $\beta$  has been shown to stimulate the secretion of PGI<sub>2</sub> in endometrial stromal cells (Levin *et al.* 1992). Whether this is associated with up-regulation in the expression of the IP receptor is unclear. Expression of the IP receptor may also be regulated by PGI<sub>2</sub> or other prostaglandins. Expression of PGIS and synthesis of PGI<sub>2</sub> is induced by COX-2 (Caughey *et al.* 2001), which is up-regulated during the time of menstruation (Jones *et al.* 1997). It is also plausible that local mediators within the endometrium may play a role in the regulation of PGI<sub>2</sub> synthesis and/or expression of its receptor. For instance, PGI<sub>2</sub> is a mediator of the protective effects of vascular endothelial growth factor (VEGF) on the vasculature (Zachary 2001) and PGI<sub>2</sub> biosynthesis is up-regulated by VEGF via ERK-mediated cytosolic phospholipase A<sub>2</sub> activation and arachidonic acid mobilisation (Zachary & Gliki 2001).

PGIS and IP receptor expression have been co-localised to multi-cellular compartments of the human endometrium. These include stromal, glandular epithelial, endothelial and smooth muscle cells. This suggests that PGI<sub>2</sub> acts in an autocrine/paracrine manner within the human endometrium to induce its cellular effects. PGIS and IP receptor immunoreactivities have been demonstrated previously in myocytes, vascular smooth muscle cells and endothelial cells in pregnant and non-pregnant human myometrium (Moonen *et al.* 1986, Chegini & Rao 1988, Giannoulas *et al.* 2002). To our knowledge, however, this the first report of localisation of PGIS and IP receptor in glandular epithelial and stromal cells within the human endometrium. Previous studies using autoradiography with [<sup>3</sup>H]PGI<sub>2</sub> on human uterine tissue failed to demonstrate PGI<sub>2</sub> binding sites in epithelial cells (Chegini & Rao 1988), although in that study binding sites were demonstrated in myometrial smooth muscle. This inconsistency with findings presented herein may reflect differences in the sensitivity of the methods used. Interestingly, stromal expression of PGIS and IP receptor was highest

in the functional layer of the endometrium. In premenopausal women, the human endometrium undergoes phases of proliferation and apoptosis during successive menstrual cycles. These phases are observed predominantly in the functional layer of the endometrium, which is shed at menstruation before regenerating during the proliferative phase of the subsequent menstrual cycle. Hence, this spatio-temporal expression of PGIS and IP receptor may be crucial for, and in keeping with, its predicted role in menstruation.

Baird *et al.* (1996) have postulated a role for PGI<sub>2</sub> in menstruation based on its myometrial smooth muscle and vascular relaxation effects and inhibition of platelet aggregation. This would counteract the effects of other prostaglandins such as PGF<sub>2α</sub>, which causes vasoconstriction and myometrial smooth muscle contraction (Crankshaw & Dyal 1994). It is also likely that PGI<sub>2</sub> is involved in the repair of the vascular bed, since it has protective effects on the endothelium by inhibiting vascular smooth muscle proliferation and enhancing endothelial cell survival (Zachary 2001). The increased expression of PGIS and IP receptor during menstruation is also consistent with a role for PGI<sub>2</sub> in the aetiology of menorrhagia. Evidence for this has been provided previously by studies of dysfunctional menstrual bleeding, which have demonstrated increased prostaglandin synthesis including PGI<sub>2</sub> (Smith *et al.* 1981*b*, Cameron *et al.* 1987) or increased synthesis of PGI<sub>2</sub> relative to thromboxane A<sub>2</sub> (Makarainen & Ylikorkala 1986*b*) in uterine tissue from women with excessive blood loss relative to controls. Whether IP receptor expression and signalling are also elevated in the endometrium of women with dysfunctional menstruation remains to be established.

In summary, this study has demonstrated temporal expression of PGIS and IP receptor in the non-pregnant human endometrium. Expression of both genes is highest during the menstrual phase and is localised to multi-cellular compartments. The function of PGI<sub>2</sub> in the human endometrium is linked to the protein kinase A pathway during menstruation. Future studies will elucidate the exact role of PGI<sub>2</sub> in menstruation and the mechanisms of its signalling in the human endometrium.

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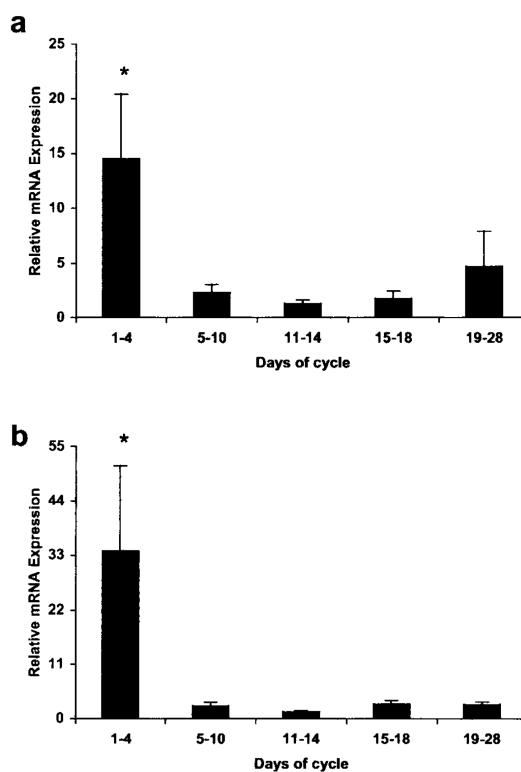
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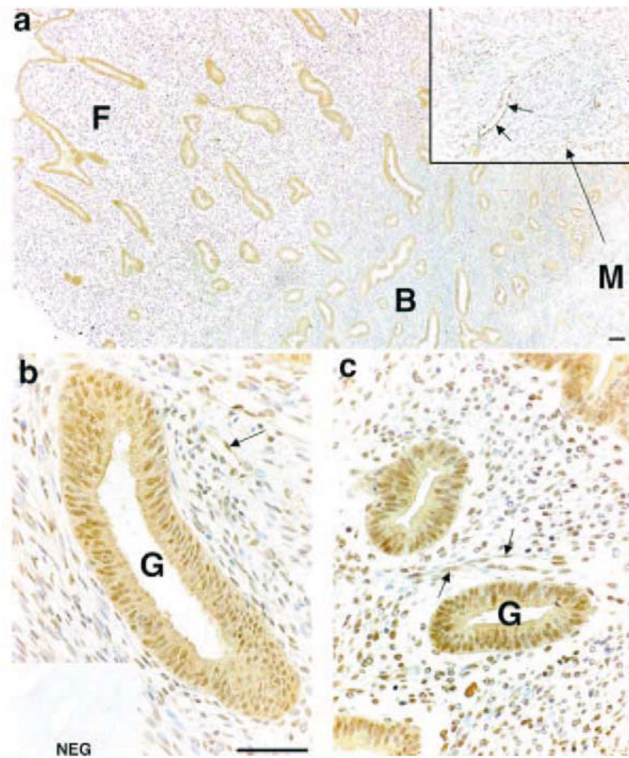


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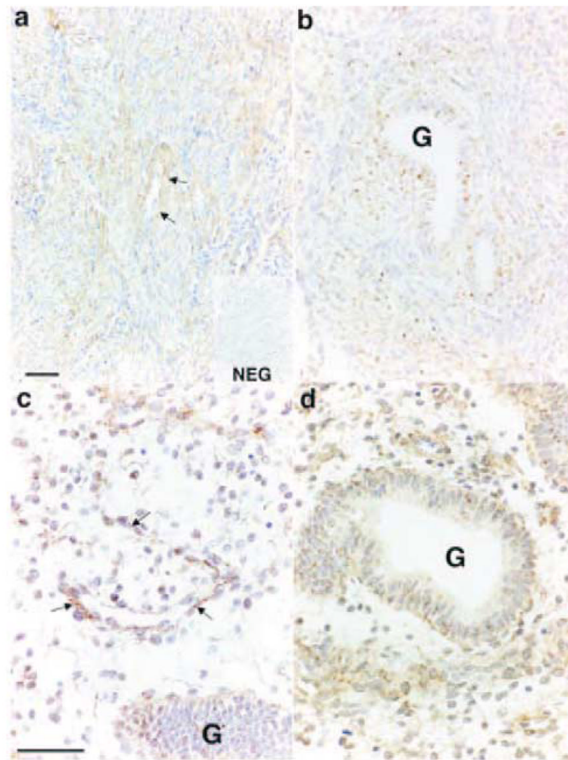


**Figure 1.** Quantitative RT-PCR of (a) PGIS and (b) IP receptor in the human endometrium across the menstrual cycle. Results are expressed as the mean  $\pm$  S.E.M. of relative mRNA expression levels. \* $P < 0.05$ .



**Figure 2.**

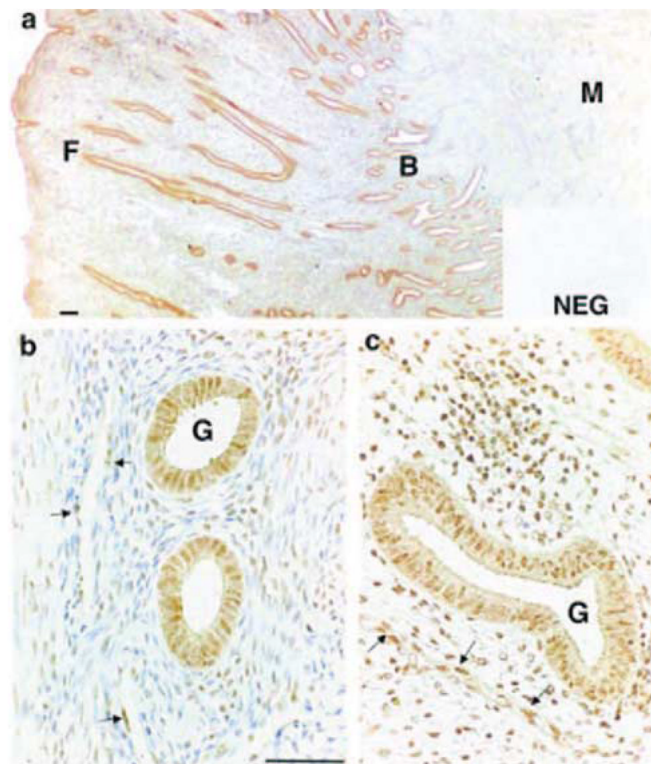
Immunohistochemical localisation of PGIS in human endometrial tissue (functional layer and basal-myometrial junction) collected in the proliferative phase of the menstrual cycle. Strong staining was detected in the glandular epithelial cells (G) in basal (B) and functional (F) layers (a-c) and in smooth muscle cells in the myometrium (M) (enlarged area in inset to a). Stromal staining was detected in the basal (b) and the functional layer (c) and endothelial cell reactivity was present in blood vessels (indicated by arrows) in all layers. NEG, negative control; scale bars = 50  $\mu\text{m}$ .



**Figure 3.**

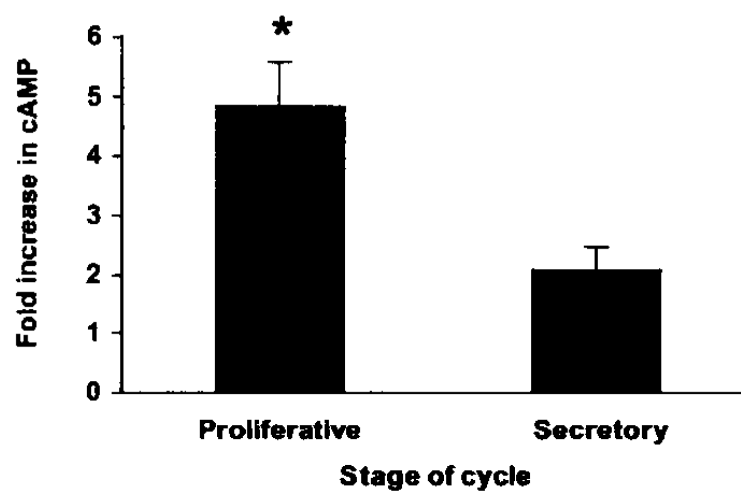
*In situ* hybridisation of IP receptor in sections of human endometrial tissue collected in the proliferative phase of the menstrual cycle. IP receptor was expressed in smooth muscle cells in the myometrium (a) and reactivity was also present in glandular epithelial cells (G) and in stromal cells in both the basal layer (b) and the functional layer (c and d). IP receptor was also expressed in vascular endothelial cells (indicated by arrows in a and c). NEG, negative control; scale bars = 50  $\mu$ m.





**Figure 4.**

Immunohistochemical localisation of IP receptor in human endometrial tissue (functional layer and basal-myometrial junction) collected in the proliferative phase of the menstrual cycle. Glandular epithelial staining (G) was present in both basal (B) and functional (F) layers and reactivity was detected in smooth muscle cells in the myometrium (M) (a-c). Stromal cell staining was present throughout the endometrium, but was stronger in the functional layer (c) compared with the basal layer (b). Endothelial cells throughout the endometrium and at the basal-myometrial junction also exhibited positive reactivity for the IP receptor (indicated by arrows in b and c). NEG, negative control; scale bars = 50  $\mu$ m.



**Figure 5.** Fold increase in cAMP generation in endometrial tissue collected from the proliferative ( $n = 4$ ) and secretory ( $n = 4$ ) phases of the menstrual cycle in response to  $100 \text{ nmol l}^{-1}$  iloprost. Results are expressed as the mean  $\pm$  S.E.M. of fold cAMP induction. \* $P < 0.05$ .